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THE EMBRYOGENY OF ZAMIA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XLV.

JOHN M. COULTER and CHARLES J. CHAMBERLAIN.

(WITH PLATES VI-VIII)

OUR knowledge of the development of the embryo of Cycadales is very meager, being largely included in Treub's (5) account of *Cycas circinalis* and Ikeno's (8) of *Cycas revoluta*. These two accounts agree in every important particular, and deal chiefly with the development of the proembryo. The following facts were established: the egg nucleus divides, and successive simultaneous divisions give rise to a large number of free nuclei; the cytoplasm of the central region of the egg becomes vacuolate and then completely disorganizes; the remaining cytoplasm becomes parietal, massing somewhat toward the bottom of the egg; in this parietal layer the numerous free nuclei are imbedded, being equidistant from one another and forming usually a single layer except at the base of the egg, where simultaneous nuclear division begins again; later, walls appear, and the proembryo becomes a sac somewhat thickened at base, but with the wall composed of one or at most two layers of cells. This history differed so much from that of Ginkgoales and of Coniferales as known at that time that it seemed to stand somewhat stiffly apart among gymnosperms.

A series of collections of *Zamia floridana* was obtained from southern Florida during the spring and summer of 1902. Some collections consisted only of the ovulate strobili, but in most cases the entire plant was secured. As stated by Webber (11), the ovulate strobili continue in their development for some time after removal from the plant, and we have observed mitotic figures in ovules after the strobilus had been lying in the laboratory for nearly two weeks. Entire plants sent from Florida in June and July were potted, and they continued to develop

quite normally, a large proportion of the ovules becoming seeds with fully formed embryos. The material yielded a fairly complete series of stages from a period somewhat before fertilization to the ripening of the seeds.

The presence of a ventral canal cell among the Cycadales was for some time in question. Strasburger (2) seems to have been the first to recognize it in 1876 in *Cycas sphaerica*, and in 1877 Warming (3) reported a ventral canal cell in *Ceratozamia robusta*, but soon (4) decided that he had been mistaken. In 1884 Treub (5) failed to find one in *Cycas circinalis*, and the impression became general that no such cell is formed among Cycadales. In 1896, however, Ikeno (6) announced that a ventral canal cell is formed in *Cycas revoluta*, and in a later paper (8) he figured and described the mitosis that separates the nuclei of the ventral canal cell and egg. In 1897 Webber (7) reported that in *Zamia* "a small cell is cut off at the apex of the archegonium, which corresponds to the canal cell of Conifers."

In our study of *Zamia* the mitotic figure was found which gives rise to the nuclei of the egg and ventral canal cell (*fig. 1*). The chromosomes are very slender and inconspicuous, and the spindle is somewhat multipolar. Considering the great size of the central cell, the whole structure is extremely small and might easily escape notice unless well stained. After the mitosis is completed the nucleus of the egg begins to enlarge and to move toward the center of the cell, while the other nucleus becomes smaller and soon disorganizes (*fig. 2*). It will be seen from *fig. 2* that there is no wall between the two nuclei, and we were not able to find any case in which a definite ventral canal cell had been cut off.

The protuberance in which the ventral canal nucleus lies rapidly disorganizes and remains for a time as a more deeply staining mass at the top of the egg. It seems probable that in his first account Warming (3) was describing as a ventral canal cell the disorganizing protuberance in which the ventral canal nucleus lies. Ikeno's *fig. 11* (8) seems to be almost identical with our *fig. 2*, and although he states that "this portion (the

protuberance) becomes completely separated from the egg cell," it is difficult to see how a wall such as usually separates the ventral canal cell from the egg could be formed after the achromatic figure has disappeared. It seems more likely that here, as in *Zamia*, the disorganizing protuberance might have been taken for a cell, and Ikeno himself in a brief note at the close of his paper on *Ginkgo* (10) admits that the wall may be lacking. In *Ginkgo*, which in so many features resembles the Cycadales, a definite ventral canal cell is separated from the egg by a wall, as figured by Strasburger (1), and the details of the mitosis are described by Ikeno (10), who figures a conspicuous cell plate at the telophase of division.

The Coniferales present some variation in the formation of the ventral canal cell; in most of the cases reported a definite wall being formed between the two nuclei, but in *Cephalotaxus* (Arnoldi, 9), in *Taxodium* and *Podocarpus* (Coker, 12), and in *Thuja* (Land, 13), no wall is formed. Arnoldi's statement that the ventral canal cell is lacking in the Cupresseae certainly does not hold for the group, and it is doubtful whether such a cell or at least a nucleus representing it is absent in any gymnosperm. It would be of interest to know whether in any of the Cycadales a definite wall is formed between the nuclei of the egg and ventral canal cell. Since such a wall is uniformly present in the bryophytes and pteridophytes, its absence among the Cycadales would have to be regarded as a case of suppression.

Our preparations of the fertilization stages in *Zamia* merely confirm the very full account recently published by Webber (11).

The immense size of the fertilized egg of *Zamia* is a matter of surprise to any who are not familiar with the archegonia of Cycadales. The fertilized egg is usually 3^{mm} in length, and its nucleus is plainly visible to the naked eye, often reaching a length of 1000 μ , as shown in *fig. 3*, which shows the actual size of the upper portion of the gametophyte with two archegonia.

After fertilization there is a period of free nuclear division. These divisions are simultaneous (*fig. 4*) and follow one another with such rapidity that the nuclei become smaller at each successive division. The first nuclear division was not found in

our preparations. Webber (11) observed the second division and states that the first two divisions occur near the center of the egg, and that the nuclei then become scattered, and that "finally in the first stage of the organization of the embryo form a layer of cells about the periphery of the egg cell." We were able to count the nuclei in the 16-nucleate and 32-nucleate stages, being of course the product of the fourth and fifth simultaneous divisions. The 64-nucleate and 128-nucleate stages were counted approximately. In two cases the mitotic figures of the eighth division, which gives rise to the 256-nucleate stage, were counted with considerable certainty, assuming, as seems safe, that these early divisions are all simultaneous. In both of these series, which consisted of more than fifty sections, from several of which the proembryo had been lost in making the preparations, over one hundred figures were counted. Since the seventh division would show only 64 figures and the ninth division 256, there could hardly be a possibility of mistaking this stage. A section from one of these series is represented in *fig. 4*.

The entire mitotic figure is formed within the nuclear membrane, which does not disappear until the anaphases are reached. The kinoplasmic portions of the figure are highly developed, the polar radiations being particularly conspicuous. The whole figure differs decidedly from the one concerned in the formation of the ventral canal nucleus (*fig. 1*), for in this case the nuclear membrane disappears before the metaphase is reached, and there are no polar radiations whatever; and also from the later divisions in the embryo, in which the nuclear membrane disappears early. That simultaneous divisions cease with the eighth division may be regarded as certain, for numerous preparations of the proembryo just before the formation of the cell wall (*figs. 5, 6*) fail to show even approximately the large number of nuclei (512) which should be found if a ninth division had taken place.

The appearance of the proembryo at the close of free nuclear division, but before the formation of any cell walls, is shown in *figs. 5 and 6*. In both these figures the nuclei are scattered throughout the entire egg, there being no tendency to form a large central vacuole with consequent parietal placing of the

nuclei, as in *Cycas*. In the stage represented in *fig. 5* the nuclei are more numerous in the lower part of the proembryo, doubtless on account of the settling of some of them. This settling is sometimes very evident, the nuclei being lined up along strands of cytoplasm, which appear almost as definite as bundles of spindle fibers, although they are only such trails as might be caused by nuclei passing rapidly through the cytoplasm. Very similar appearances were noticed in eggs which had been ruptured by the pressure of the razor in cases where the portion containing the archegonia had been cut out previous to placing in the fixing fluid. In these cases the trails were caused by the rapid movement of the cytoplasm toward the point of exit.

It is worthy of note that in most of our preparations of the early stages, including the eighth simultaneous nuclear division (*fig. 4*), the nuclei are nearly all in the lower half of the egg; while in later stages (*figs. 5-10*) the upper portion is never free from nuclei. In this connection it should be noted also that just before the appearance of cell walls (*figs. 5-6*) the number of nuclei is often more than 256, although never so many as 512, the number which would result from a ninth simultaneous division. It seems probable that many of the nuclei in the upper part of the proembryo, which is not to form any part of the embryo proper, are due to irregular or amitotic division. It is certain that the nuclei of this region are irregular in size and often amoeboid in outline. Some of these nuclei may have been due to the entrance of two male cells, one remaining near the apex and dividing. The two large irregular nuclei shown in *fig. 4* suggest this explanation. While the ventral canal nucleus might divide and give rise to nuclei in this region, we have seen nothing which would make such an explanation probable.

Ikeno's (8) account indicates that even in the earlier stages of development the embryo of *Cycas* differs considerably from that of *Zamia*. In *Cycas*, as in *Zamia*, fertilization is followed by a period of free nuclear division, but in *Cycas circinalis* (Treub, 5) and also in *C. revoluta* (Ikeno, 8) a large central vacuole is soon formed. According to Ikeno, who gives a more detailed account, the nuclear division near the neck of the

archegonium may be amitotic; after the number of free nuclei has become quite large, the vacuole is formed by the disorganization of the cytoplasm and free nuclei of the central portion of the egg. After the vacuole has become established there remains at the base of the egg a rather dense mass of cytoplasm containing numerous free nuclei, while the cytoplasm in other parts of the egg is reduced to a thin peripheral layer with comparatively few nuclei. At this period free simultaneous nuclear division is resumed in the region at the base of the egg, which is apparently quite sharply marked off (Ikeno, 8, fig. 49), so that there are two rather definite periods of free nuclear division. In *Zamia* (figs. 3, 5, 10) there is only one period of free nuclear division, and no vacuole is formed during these early stages or even after the suspensor has begun to elongate, the free nuclei of the upper portion being scattered throughout the cytoplasm. Even in stages more advanced than that shown in fig. 10 a vacuole has not yet formed, although the cytoplasm is becoming very scanty in the region which in *Cycas* is occupied by the large vacuole. Of course in later stages of development (fig. 11) the entire region, which in fig. 10 is occupied by cytoplasm and free nuclei, becomes empty.

In the formation of cell walls also *Zamia* differs from *Cycas*. In *Cycas circinalis* walls are formed throughout the entire region occupied by nuclei, while in *Zamia* the formation of walls is restricted to rather a limited area at the base of the egg. The earlier walls (fig. 7) are formed as in the endosperm of those angiosperms in which a period of free nuclear division precedes the formation of cell walls, excepting that in *Zamia* we have never found more than one nucleus in a cell, as sometimes occurs in angiosperms. At first the cells are all of the same size, but very soon those at the base begin to stain more deeply, and on account of more rapid division become smaller than those above them (fig. 8). The differentiation soon becomes quite marked, and the elongating cells of the suspensor can be distinguished easily from those of the embryo proper (fig. 9). In the last-cited figure, as well as in figs. 8 and 10, it may be seen that just above the definitely walled cells there is a region where

wall formation is indefinite and incomplete, and that beyond this no walls are formed.

The rapid elongation of the suspensor (*figs. 13-16*) forces the embryo down into the endosperm. The resistance is so great that in later stages the base of the embryo is forced upward into the archegonial chamber and is stopped only by the hard seed coat. The suspensor is long and twisted. When straightened out (*fig. 11*) it may be fully 5^{cm} long.

Certain features of the mature embryo may mentioned. The two cotyledons are free at apex and base, but are more or less completely fused in the middle region. The long cotyledons (*fig. 12*) constitute much the larger part of the embryo, the whole axis being comparatively small. The plumule is well developed, several leaf primordia being evident. Several branching mucilage canals extend from the root to the tips of the cotyledons. According to Warming (4) the embryo of Ceratozamia has only one cotyledon, a part of the meristematic apex of the axis becoming differentiated into the single cotyledon and the rest forming the stem tip.

These results with *Zamia* have enabled us to arrange a suggestive series of gymnosperms as shown by the accompanying diagram (*fig. 17*). In the first series a gradual reduction in the number of free nuclei is shown. In *Ginkgo*, *Cycas*(?), and *Zamia* it would seem that eight successive simultaneous nuclear divisions precede wall formation, resulting in 256 free nuclei. Among the Coniferales there is a sudden drop in the number of free nuclei, but at the same time a diminishing number from 32 to 4, as follows: *Taxus* 32; *Cephalotaxus*, *Podocarpus*, and *Taxodium* 16; *Thuja* 8; *Pinus* 4. While these numbers probably represent the usual condition, the higher numbers are not entirely constant, wall-formation sometimes occurring at an earlier division than usual. For example, in *Podocarpus* wall-formation may occur after the third division, but usually only after the fourth; while in *Taxus* wall-formation has never been observed to occur until after the fourth division, and often only after the fifth. Further reduction in the number of free nuclei occurs among the Gnetales, *Ephedra* sometimes showing only a

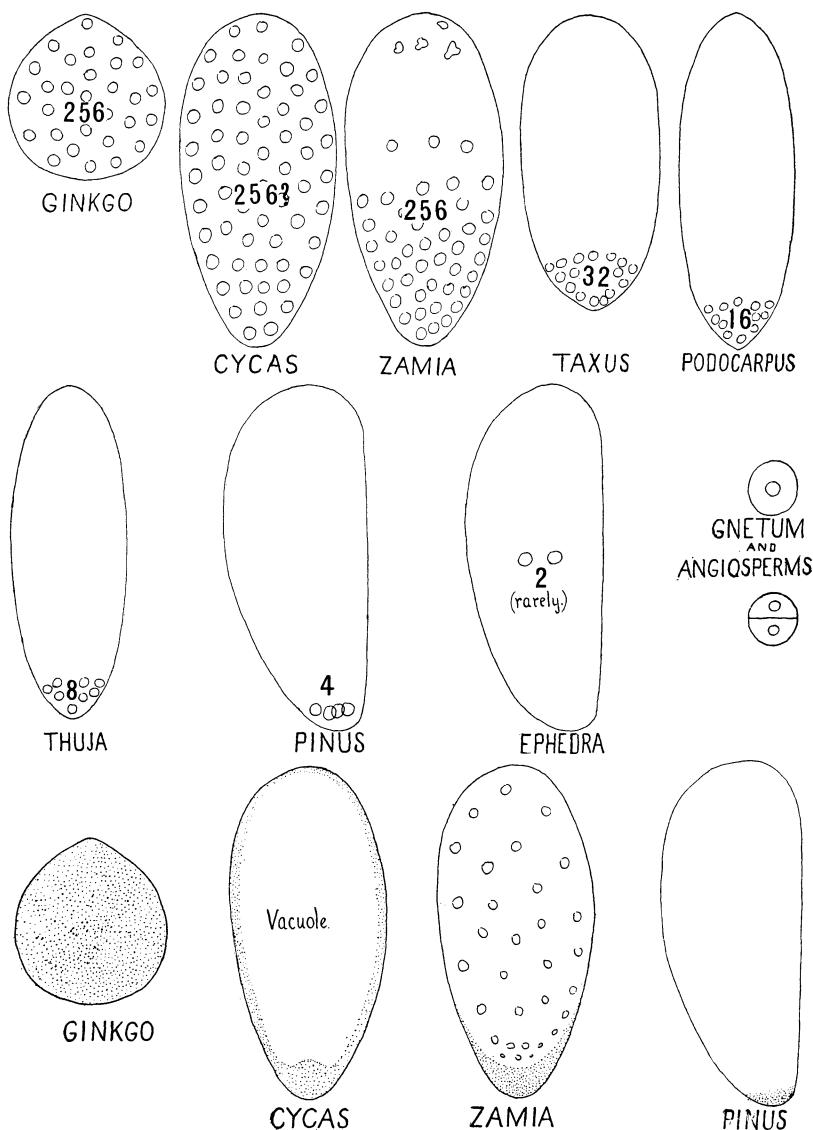


FIG. 17. A series of diagrams to illustrate the extent of free nuclear division and wall-formation; the shaded portions of the last four figures represent the region of wall-formation.

single free nuclear division, resulting in two free nuclei; and in *Gnetum* and *Tumboa* no free nuclear division occurs at all. These last-named forms have reached the condition of the embryogeny of angiosperms, in which the first division of the nucleus of the egg is accompanied by the formation of a wall. The gap between *Zamia* with its 256 free nuclei and *Taxus* with its 32 may seem great at first sight; but it should be noted that it represents the omission of only two successive divisions, the sixth and seventh. It is entirely possible, therefore, that these missing numbers (64 and 128) may be found among the numerous Cycadales and Coniferales that await investigation.

In the second series shown in the diagram a later stage is presented. In this series *Ginkgo* is clearly at one extremity with its numerous free nuclei equally distributed, and its wall formation resulting in a proembryo that completely fills the egg. In *Cycas* the free nuclei do not remain evenly distributed, but there is a massing toward the base of the egg, and most of the remaining nuclei through the formation of a central vacuole pass to the periphery and enter into the formation of a parietal layer. In *Zamia* wall-formation is still further restricted, appearing only in connection with the basal nuclei. There is also no development of a central vacuole, and hence no parietal placing of free nuclei. In *Zamia*, therefore, a tissue that fills the sac, as in *Ginkgo*, or that lines the egg, as in *Cycas*, is reduced, and its formation is restricted to the basal region, as in Coniferales. In passing from this condition, represented by *Zamia*, to the condition displayed by the Coniferales, there is a gradual reduction in the number of nuclei that enter into the formation of this basal tissue, and as a result a gradually more definite setting apart of the function of each particular nucleus. For example, in *Zamia* the suspensor cells are not recognizable until they begin to elongate; in *Taxus* with its 32 nuclei, and in *Cephalotaxus*, *Podocarpus*, and *Taxodium* with their 16, there is no setting apart of a distinct suspensor-forming layer; while in *Thuja* with its 8 nuclei a distinct suspensor-forming layer is recognized, and this definiteness is continued into the 4-nucleate forms.

It would seem to follow from these considerations that the

embryogeny of Ginkgo is the most primitive one among gymnosperms; that the embryogeny of Cycas is more primitive than that of Zamia; that in its embryogeny Zamia approaches more nearly the Coniferales than does Cycas; that such forms as Taxus, Cephalotaxus, Podocarpus, Taxodium, and Thuja show progressive stages of embryogeny from that of Zamia toward that of Pinus; that Ephedra has the most primitive embryogeny among the Gnetales; and that Gnetum and Tumboa resemble the angiosperms in the elimination of free nuclear division from their embryogeny.

UNIVERSITY OF CHICAGO.

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EXPLANATION OF PLATES VI-VIII.

All the figures are from *Zamia floridana*. *Figs. 13-16* are from photomicrographs of rather thick sections. The rest of the figures, except *figs. 3, 11, 12*, are from camera lucida drawings.

FIG. 1. The mitotic figure which gives rise to the nuclei of the egg and the ventral canal cell. $\times 200$.

FIG. 2. A later stage showing the two neck cells (*a*), the ventral canal nucleus (*v*), and the nucleus of the egg (*e*). $\times 45$.

FIG. 3. Upper portion of the gametophyte showing two archegonia; the nucleus of the fertilized egg (*n*) is plainly visible to the naked eye. *Natural size*.

FIG. 4. Free simultaneous nuclear division in the proembryo; the number of mitotic figures in this proembryo is estimated at 128; the two large irregular nuclei at the top may have come from a male cell; the spindle-shaped bodies at the top are probably coagulated food material. $\times 25$.

FIG. 5. Proembryo after simultaneous free nuclear division has ceased; the remains of the blepharoplast (*b*) can still be seen. $\times 20$.

FIG. 6. A stage slightly later than that shown in *fig. 5*; the strands at the bottom seem to be related to the settling of the nuclei. $\times 20$.

FIG. 7. The beginning of wall-formation at the base of the proembryo. $\times 20$.

FIG. 8. Differentiation into suspensor and embryo proper becoming distinguishable. $\times 30$.

FIG. 9. A more advanced stage, the embryo and suspensor being quite definitely outlined; the faint and incomplete septation above the suspensor is also shown. $\times 50$.

FIG. 10. A still later stage; the suspensor has lengthened considerably; no central vacuole has yet been formed, although the cytoplasm in the region occupied by the vacuole in *Cycas* at this stage and earlier is becoming scanty. $\times 36$.

FIG. 11. Young embryo dissected out from the seed; the suspensor has been pulled out straight. *Natural size*.

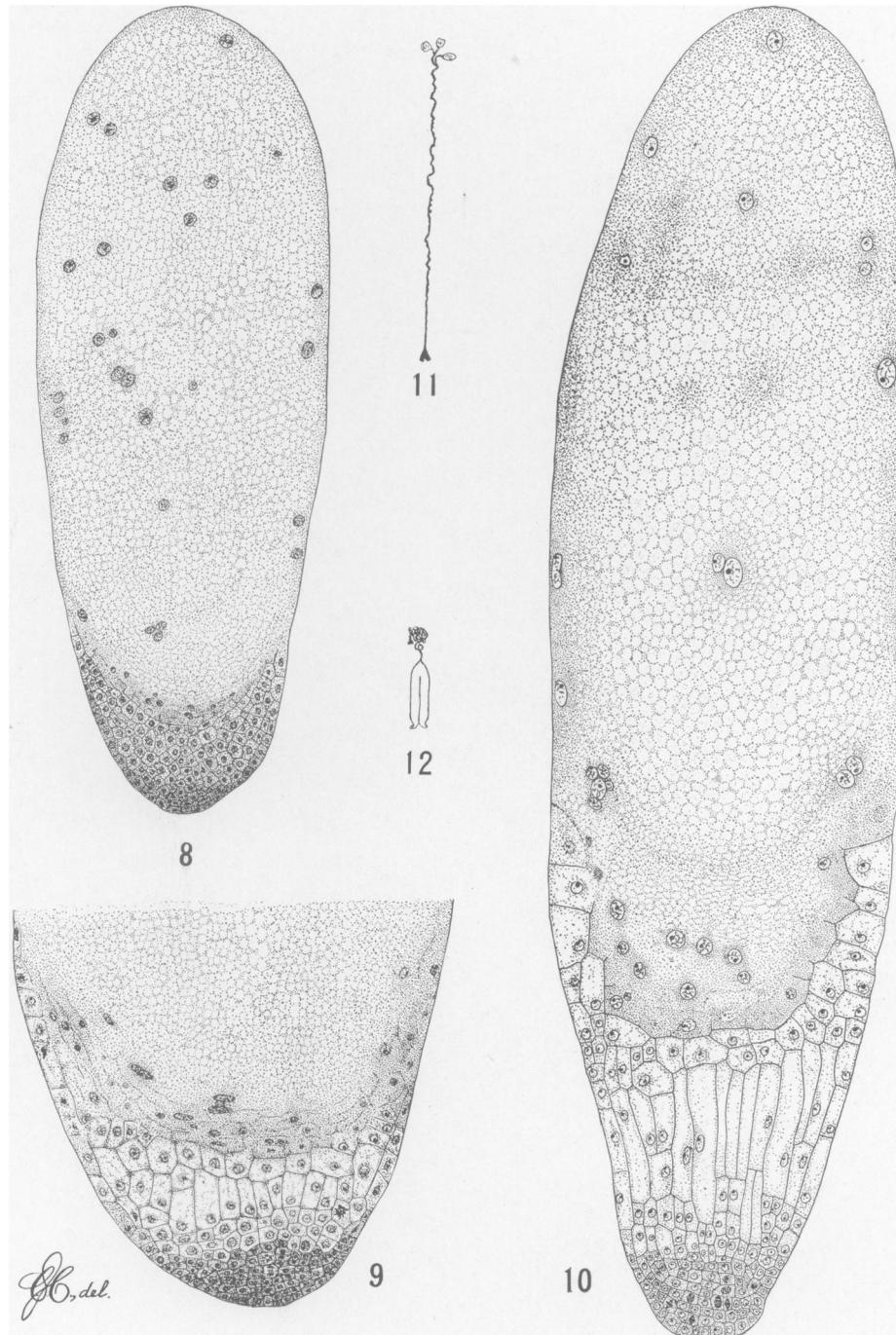
FIG. 12. A nearly mature embryo, showing the two cotyledons and suspensor. *Natural size*.

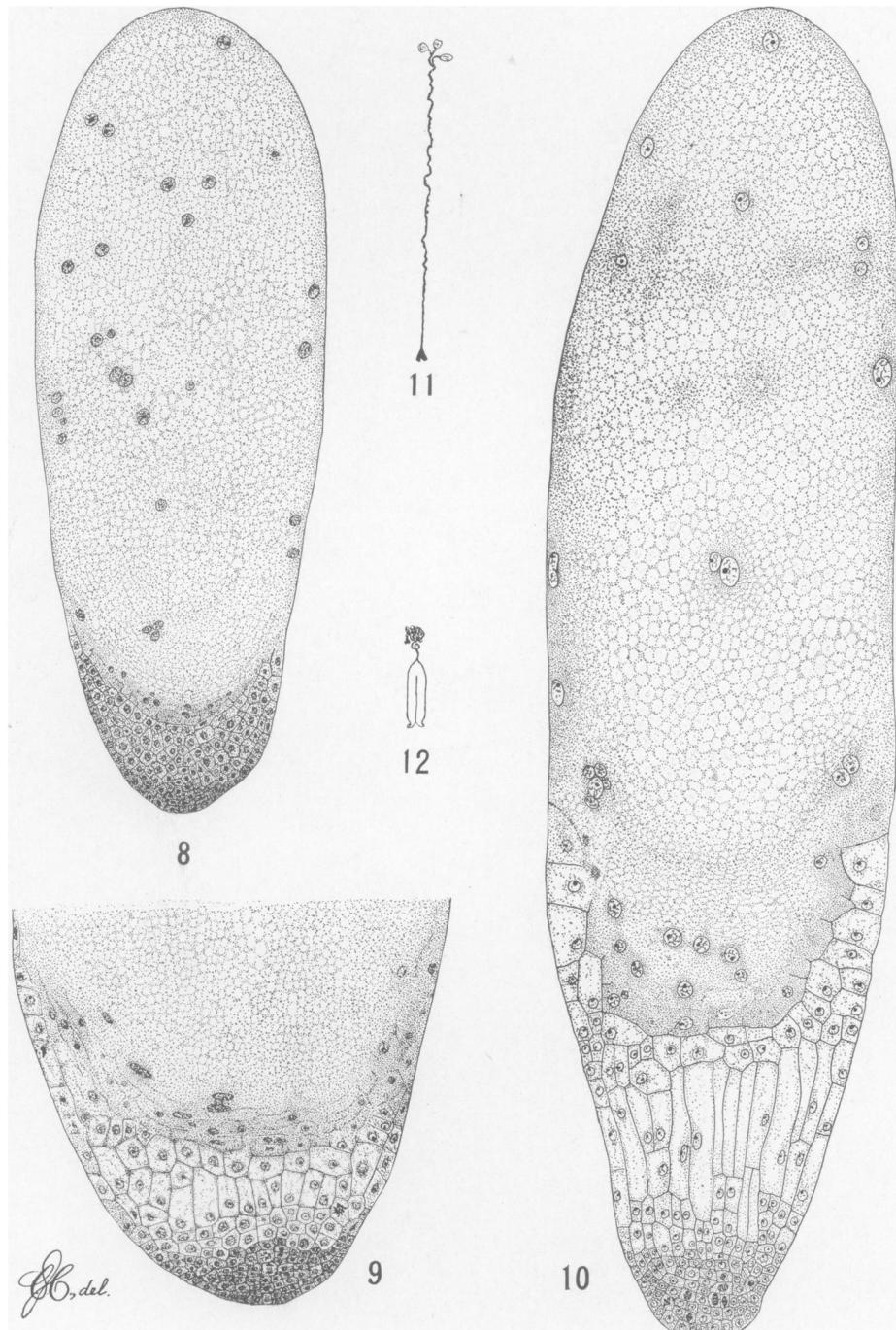
FIG. 13. Upper part of gametophyte showing embryo and characteristic cavity formed by disintegration of surrounding tissues. $\times 14$.

Fig. 14. Three embryos in about the same stage as that shown in the preceding figure. $\times 45$.

FIG. 15. Tip of young embryo pressing into the endosperm. $\times 185$.

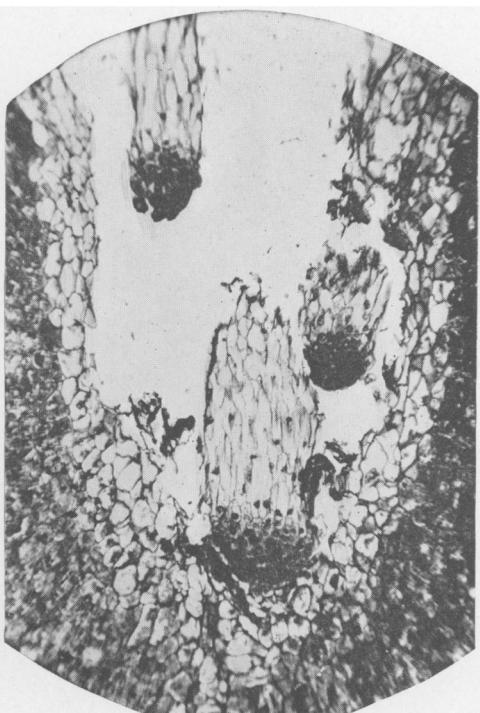
FIG. 16. Young embryo with a portion of the suspensor. $\times 140$.



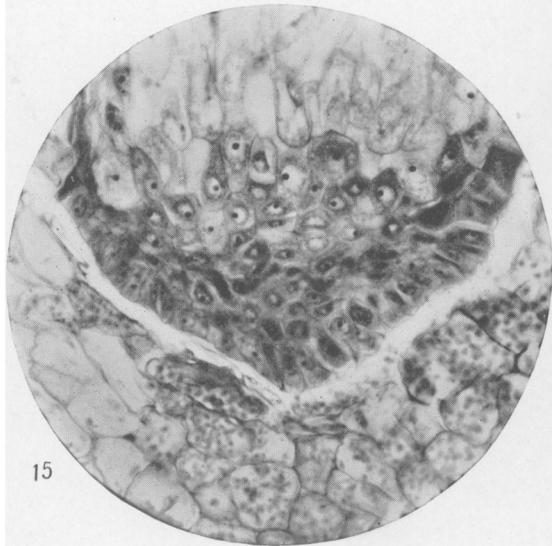




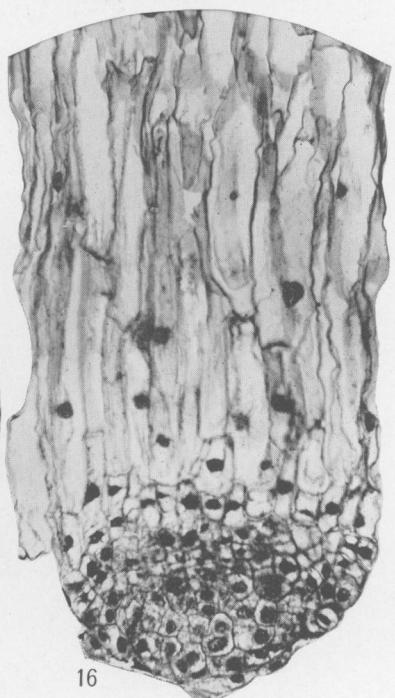
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